

AWARD NUMBER: W81XWH-14-1-0401

TITLE: Topical Modulation of the Burn Wound Inflammatory Response to Improve Short and Long Term Outcomes

PRINCIPAL INVESTIGATOR: Saman Arbabi, MD, MPS

CONTRACTING ORGANIZATION: University of Washington
Seattle, WA 98195

REPORT DATE: October 2016

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE**Form Approved**
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Service, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188) Washington, DC 20503.

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**1. REPORT DATE (DD-MM-YYYY)**

October 2016

2. REPORT TYPE

Annual

3. DATES COVERED (From - To)

15 Sep 2015 -14 Sep 2016

4. TITLE AND SUBTITLE

Topical Modulation of the Burn Wound Inflammatory Response to Improve Short and Long Term Outcomes

5a. CONTRACT NUMBER**5b. GRANT NUMBER**

W81XWH-14-1-0401

5c. PROGRAM ELEMENT NUMBER**6. AUTHOR(S)**

Dr. Saman Arbabi

email: sarbabi@uw.edu

5d. PROJECT NUMBER**5e. TASK NUMBER****5f. WORK UNIT NUMBER****7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

University of Washington Office of Sponsored Programs

4333 Brooklyn Ave NE

Box 359472

Seattle, WA 98195-9472

**8. PERFORMING ORGANIZATION
REPORT NUMBER****9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

U.S. Army Medical Research and Materiel Command

Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)**11. SPONSORING/MONITORING
AGENCY REPORT NUMBER****12. DISTRIBUTION AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES**14. ABSTRACT**

We propose to investigate the relationship between p38MAPK signaling, wound inflammatory response, wound healing and long-term scar formation using a burn model in the female red Duroc pig. We hypothesize that topical p38MAPK inhibition will attenuate the depth of the burn by preventing hair-follicle cell apoptosis, attenuate the inflammatory phase of wound healing, and decrease the granulation layer thickness. We propose this modification in the early inflammatory response will also reduce thickness and contraction of scars formed after deep partial thickness burn injury. The knowledge gained from our proposed research will be critical to implement a potential paradigm shift in the clinical treatment of challenging dermal injuries.

15. SUBJECT TERMS

None listed

16. SECURITY CLASSIFICATION OF:**a. REPORT**
Unclassified**b. ABSTRACT**
Unclassified**c. THIS PAGE**
Unclassified**17. LIMITATION OF
ABSTRACT**
Unclassified**18. NUMBER
OF PAGES**

14

19a. NAME OF RESPONSIBLE PERSON
USAMRMC**19b. TELEPHONE NUMBER (Include area code)**

Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	4
3. Accomplishments.....	4
4. Impact.....	11
5. Changes/Problems.....	11
6. Products.....	12
7. Participants & Other Collaborating Organizations.....	12
8. Special Reporting Requirements.....	13
9. Appendices.....	13

1. INTRODUCTION

Approximately 500,000 Americans suffer burn injuries with an estimated 3,500 deaths annually. Widespread makeshift bombs contribute to burns and large wounds being one of the significant causes of warfighter casualties. The magnitude and impact of burns can be devastating as large numbers casualties occur simultaneously. Secondary organ damage and failure frequently occurs after injury. Moreover, wound complications such as hypertrophic scars may cause significant morbidity, disabling loss of function, extended difficult recovery times, dramatically affecting the patient's quality of life physically. We are investigating a topical therapy that is easy to apply and can be used by a wider range of health care providers in a mass-casualty incident.

2. KEYWORDS:

Wounds, Burn, topical, wound healing, inflammatory signaling, Mitogen activated protein kinase, hypertrophic scar, p38, combat casualty, treatment, organ failure, systemic inflammatory response syndrome, thermal injury, wound model, intervention

3. ACCOMPLISHMENTS:

What were the major goals of the project?

1. Establish the female red Duroc pig model burn model as the appropriate wound healing model that resembles human response. At the end of the project, we will have a well-defined animal model for human wound healing. This animal model may provide a tool that other investigators can use to screen for compounds that may modify wound healing and reduce scar formation. The ability to have a standard animal model for wound healing may bring an exciting new era in the investigation and elucidating the molecular mechanisms of hypertrophic scar pathophysiology and developing therapeutic agents.
2. Define the inflammatory signaling post burn injury and elucidate the relationship between early inflammatory signaling, wound healing, and scar formation.
3. Define the role of p38MAPK in wound healing and scar formation.
4. Identify the wound healing response to topical p38MAPK inhibition. Demonstrate early wound healing and reduced scar formation with topical p38MAPK inhibition. Define the long-term wound outcome of topical p38MAPK inhibition post-burn injury.
5. Identify the optimal timing and duration of treatment for p38MAPK therapy.
6. By the end of the project, have a well-defined protocol and experimental plan to initiate human subject research to study topical p38MAPK inhibition as a therapy to decrease end-organ dysfunction, improve wound healing, and reduce scar formation in patients with burn injuries.

What was accomplished under these goals?

Goal 1. Establish the female red Duroc pig model as the appropriate wound healing model.

The first goal of the project was to establish the female red Duroc pig model burn model as the appropriate wound healing model that resembles human response. Our first 3 porcine experiments, porcine group (Pg) 001-003, were the dermatome model, demonstrating that the female red Duroc porcine model significantly correlates to human hypertrophic scarring. We started our experiments using the burn wound model with Pg004. In this model we use a 'hot water bottle' thermal injury device. Briefly, we use a 500 ml Pyrex laboratory Schott Duran bottle with the bottom glass removed, edges smoothed, bottom replaced with cling wrap, and secured with heat resistant tape. The bottles will be filled with 300 ml of water and then heated to the desired temperature of 92°C. We have improved our technique significantly over the period of the current grant. The initial wounds were not uniform. Starting with Pg 007 (Table 1), we have resolved the technical issues and burn wounds are uniform (Figure 1). We change the depth of the burn by changing the length of contact 10, 15, and 20 seconds. We have identified that all these contact times are in the range of partial thickness injury. The 20 seconds is mostly very deep partial thickness burns with central area of full-thickness injury. Goal 1 is accomplished during the year 1 period September 2014- September 2015.



Goal 2: Define Inflammatory Signaling Post Burn Injury and Elucidate Relationship...

The second goal of the grant was to define the inflammatory signaling post burn injury and elucidate the relationship between early inflammatory signaling, wound healing, and scar formation. Rather than systemic modulation of the inflammatory response, we propose a novel approach, which calls for "inflammatory source control". We define "inflammatory source control" to be all the maneuvers that can be used to control a focus of inflammation, which is thought to be the initial source of systemic immune activation. In our burn model, we control the source of inflammation by application of a topical p38 MAPK inhibitor. The p38 MAPK pathway is the key inflammatory intracellular signaling in mammalian cells. In our previous murine models, we demonstrated that topical application of p38MAPK inhibitors after burn injury attenuated wound inflammatory response and stress signaling, leading to reduced systemic inflammatory activation and end-organ dysfunction (these data already published and not part of the current investigation). In our porcine model, our goal is to demonstrate that topical p38 MAPK inhibitors will attenuate wound inflammation and reduce scarring in the red Duroc pig model of fibroproliferative scarring. We have done total of 9 pig experiments. The porcine group experiments are numbered 001-009 (Table 1).

Goals 2 (define the inflammatory signaling post burn injury and elucidate the relationship between early inflammatory signaling, wound healing, and scar formation) and 3 (define the role of p38MAPK in wound healing and scar formation) are in progress.

Table 1					
Study ID	Wound	Dates	Treatment	Pigs #	Duration of the study
Pg001	Dermatome	Feb-10	Topical p38MAPK inhibitor versus control	2	20 weeks
Pg002	Dermatome	Sep-11	Topical p38MAPK inhibitor versus control	2	20 weeks
Pg003	Dermatome	Sept 2012-Oct 2012	PGE2 agonist topical versus control	6	3 weeks
Pg004	Scald Bottle Burn	Sept 2013- Oct 2013	Topical p38MAPK inhibitor versus control	6	2 weeks
Pg005	Scald Bottle Burn	May 2014	Topical p38MAPK inhibitor versus control	6	2 weeks
Pg006	Scald Bottle Burn	Dec 2014	Topical p38MAPK inhibitor versus control	6	3 days
Pg007	Scald Bottle Burn	May 2015	Topical p38MAPK inhibitor versus control	6	3 days
Pg008	Scald Bottle Burn	Sept-Oct 2015	Topical p38MAPK inhibitor versus control	8	2 weeks
Pg009	Scald Bottle Burn	May 2016	Topical p38MAPK inhibitor versus control	8	3 days

We have analyzed these wounds using several different methods:

- Wound character: time to wound closure, color, wound infection
- Histopathology: H&E, TUNNEL assay,
- Inflammatory and wound healing gene expression
- Custom porcine RT-qPCR Array

Table 2 demonstrates all completed, in-progress, and pending analyses (N/A is not applicable; meaning that set of analysis will not be done; for instance, no wound closure analysis will be done in 3 day experiments).

We have used porcine RT-qPCR Array for wound healing, inflammatory, and apoptosis pathways. We have demonstrated a difference in pattern of pathway expression between the burn versus non-burn skin and burn treated with p38 inhibitor versus vehicle. In the burn wound healing, there is a portion of collagen arrangement that remains intact. In the dermatome model the line between “normal” collagen and “abnormal “collagen” is sharp and clear. In the burn model there is a large transition zone between the intact dermal architecture and damaged skin. This reflects the ongoing inflammation and apoptosis seen in burn injury. When we examined the gene expression differences in various depth of injury, an interesting pattern was observed. The deeper burns were associated with increasing number of over-expression of the regulatory genes. This internal consistency in the model is very important.

Figure 2 demonstrates a heat map difference between the burn and non-burn gene expression. The figure is using Pg008 and Pg009; therefore, we can examine the gene expression after 3 days (Pg009, 72 hours) and after two weeks (Pg008, 2 weeks). The green in heat map is low activity and red is high activity. After burn injury, there is an immediate shut down of series of genes with upregulation of inflammatory and apoptotic genes. After 2 weeks the wounds have epithelialized and many of the gene expression have returned to normal.

Figure 3 demonstrates the heat map differences between treated (topical p38 MAPK inhibitor) and not treated (vehicle) after two weeks. The three groups are uninjured, burn with vehicle treatment, and burn with p38 MAPK inhibitor treatment. One pig in 20 second Vehicle is out of range and will not be used. We were expecting that the treatment group will have deactivation of set of genes in 2 weeks compared to vehicle. This is seen in certain genes such as GAPDH and BAG3. However, the data demonstrated that the treatment has more activation of a set of regulatory genes as compared to vehicle. We have not completed these analyses and the ultimate pathway analyses is pending.

Goal 3: Define the role of p38MAPK in wound healing and scar formation.

Referring to table 2, a significant portion of goal 2 (Define the inflammatory signaling post burn injury and elucidate the relationship between early inflammatory signaling, wound healing, and scar formation) and goal 3 (Define the role of p38MAPK in wound healing and scar formation) has been done. However, final analyses and completion of work is pending.

Table 2	Assay Status							
Study ID	H & E Slides	Wound Closure Images	Itch Score	Wound Contracti on Images	Wound Healing Profiler RT2-qPCR Array	Apoptosis Profiler RT2-qPCR Array	Cleaved Caspase3 (CC3) Immuno Histo chemistry	Western Blot: p38 MAPK activation, and CC3
Pg001	Completed- Analysis	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Pg002	Completed- Analysis	n/a	n/a	Completed - Analysis	n/a	n/a	n/a	n/a
Pg003	Completed- Analysis	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Pg004	Completed- Analysis	Completed- Analysis	n/a	n/a	Completed- Analysis	n/a	n/a	n/a
Pg005	Completed- Analysis	Completed- Analysis	subjective inconclusive	n/a	Completed- Analysis	n/a	n/a	n/a
Pg006	Completed- bx tissue processed thru H&E digital images. On hold- image measurements. Pending- western p38 activation results.	n/a	n/a	n/a	Completed- bx tissue biopulverized. On hold- pending western results	Completed- bx tissue biopulverized. On hold- pending western results	Completed- bx tissue processed thru slides On hold-pending western CC3 results	In progress
Pg007	Completed- bx tissue processed thru H&E digital images. On hold- image measurements. Pending- western p38 activation results.	n/a	n/a	n/a	Completed- Arrays. Completed- partial analysis (pending Pg009 arrays completion)	Completed- Arrays. Completed- partial analysis (pending Pg009 arrays completion)	Completed-bx tissue processed thru CC3 IHC digital images. Completed-RO1 analysis Completed- algorithm consult/design NWBS for R01 depth analysis On hold pending- western CC3 results	In progress
Pg008	Completed bx tissue processed thru H&E digital images. In progress- image measurements.		n/a	n/a	Completed- Arrays. Pending- final analysis formatting	n/a	n/a	n/a
Pg009	Completed bx tissue processed thru H&E digital images. On hold- image measurements. Pending western p38 activation results	n/a	n/a	n/a	Completed-bx tissue biopulverized In progress- arrays	Completed-bx tissue biopulverized In progress- arrays	Completed- bx tissue processed thru slides On hold-pending western CC3 results	In progress

Figure 2

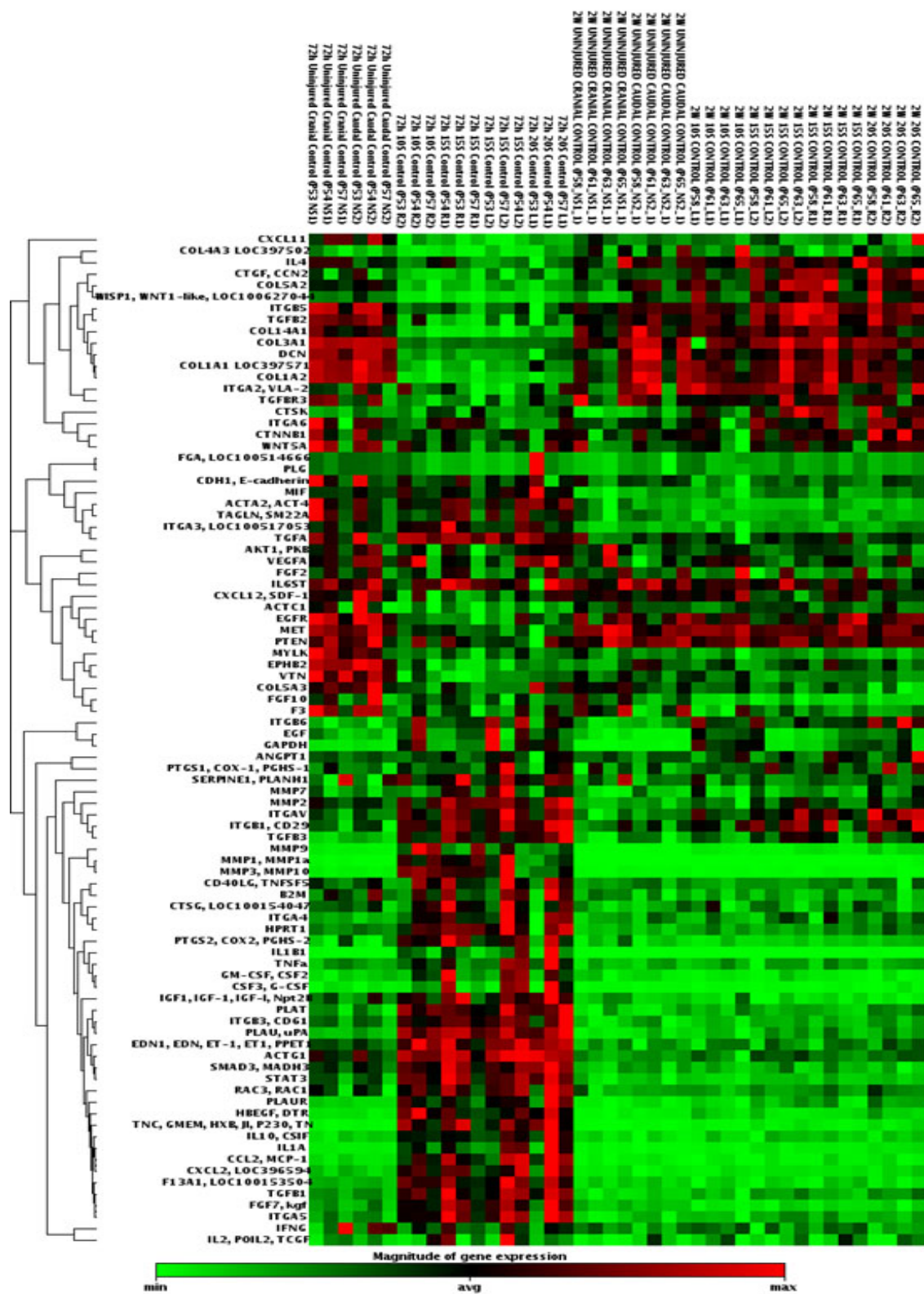
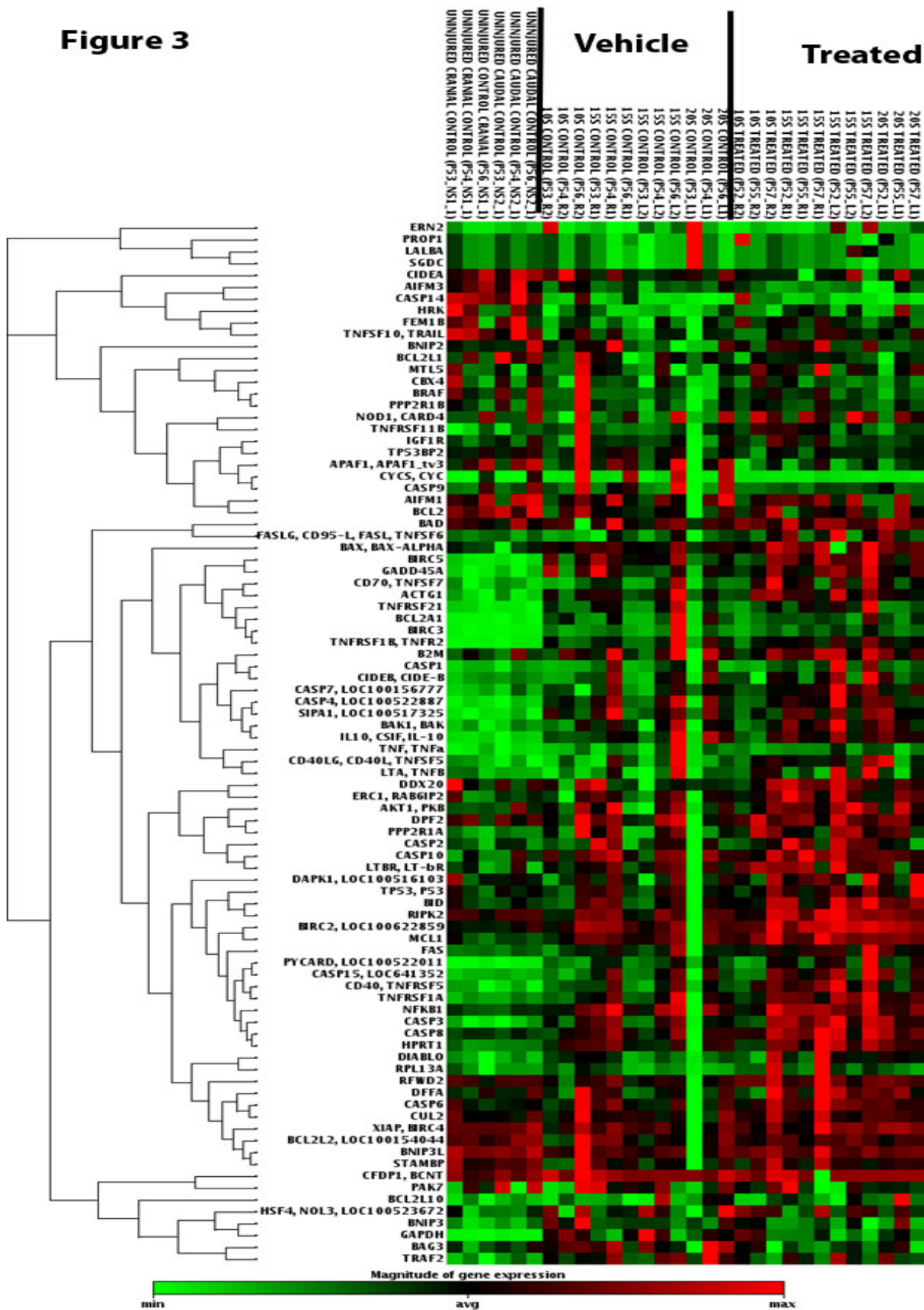


Figure 3



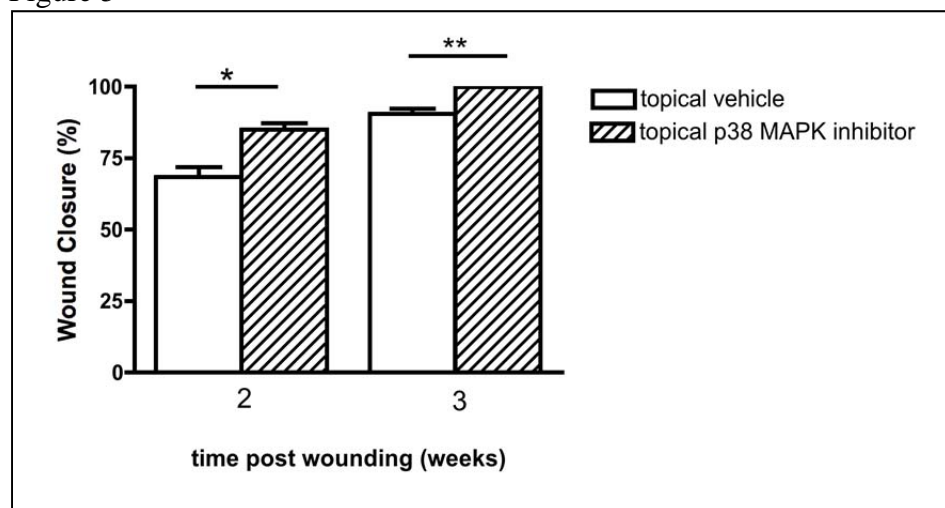
Goal 4: Identify the wound healing response to topical p38MAPK inhibition. Demonstrate early wound healing and reduced scar formation with topical p38MAPK inhibition. Define the long-term wound outcome of topical p38MAPK inhibition post-burn injury.

We have demonstrated early wound closure with p38 MAPK inhibition. Using the dermatome model in Pg001 and Pg002, we demonstrated that wounds treated with p38MAPK inhibitor epithelialized faster than control group in 2 and 3 week time-points. Pictures of each wound were analyzed by computer and the area that were epithelialized were measured (Figure 4). The data demonstrated that there was statistical significant closure rate in wounds treated with p38MAPK inhibitor (Figure 5). The burn wound data is collected and the analyses is pending. The scar formation portion of this goal is pending.

Figure 4: Percentage closure was calculated for each individual wound



Figure 5



Goal 5: Identify the optimal timing and duration of treatment for p38MAPK therapy

The duration of p38 MAPK treatment has been set at 72 hours. We have used topical p38 MAPK upto 3 days after injury. There has been no increase in wound infection or delayed wound closure. Therefore, we concluded that 72 hour time point remains optimal. It remains to be seen, if longer time is associated with better outcomes. This goal is accomplished.

Goal 6: By the end of the project, have a well-defined protocol and experimental plan to initiate human subject research to study topical p38MAPK inhibition as a therapy to decrease end-organ dysfunction, improve wound healing, and reduce scar formation in patients with burn injuries.

This is the final goal of the project. We are hoping by the end of the project to develop a human subjects study protocol. This goal is pending.

What opportunities for training and professional development has the project provided?

The collaboration with NWBiospecimen (Univ. of WA) has improved the understanding of apoptotic response in burn injury with both groups.

How were the results disseminated to communities of interest?

There were two presentations at University of Washington, presentation at the Burn annual symposium

What do you plan to do during the next reporting period to accomplish the goals?

Continue with analyses of the data, including the gene expression, apoptosis, and protein expression

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

We are learning about expressions of genes after burns. More importantly, the reduction of normal function in cells is very interesting.

What was the impact on other disciplines?

The impact of understanding inflammatory response and dermal pathology may be important in treatment of dermatological pathology, such as psoriasis.

What was the impact on technology transfer? Nothing to report

What was the impact on society beyond science and technology?

I hope in the future, we can develop a cream for burn victims.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

None

Actual or anticipated problems or delays and actions or plans to resolve them

None

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

As previously reported and already approved in the quarterly reports, we have changed the number of pigs and reduce the number. Based on the data, it appears that there is no need to have 24 hours treatment time, since pigs with 72 hours treatment time demonstrated no side effect. This will decrease the number of pigs for AIM 1 and 2 to 44 pigs (previous number was 72 pigs). Aim 3 pig numbers stay the same (8 pigs); therefore, the total pig number will be reduced to 52 for the entire 4 years (reduced from 80). Also the data at 2 weeks is similar to 3 weeks, so we are going to decrease the experimental time from 3 weeks to 2 weeks. The following table is modification of our table 1 in the grant. There is no 24 hours in the treatment frequency and no sacrifice in post-burn day 1.

Table 3	Groups	
	Group 1	Group 2
Burn	Yes	Yes
Number of Burns	3 wounds each side	3 wounds each side

Burn Time	92°C for 10, 15, and 20 sec	92°C for 10, 15, and 20 sec
Treatment	Topical Vehicle	Topical p38 inhibitor
Treatment Frequency	BID topical application	BID topical application
Treatment Duration	72 hours	
	5 days	
Sacrifice	Post-burn day 3	
	Post-burn day 7	
	2 weeks post burn	

Significant changes in use or care of human subjects. Not applicable

Significant changes in use or care of vertebrate animals. No change

Significant changes in use of biohazards and/or select agents. No

6. PRODUCTS:

No products

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Saman Arbabi, MD, MPH

Project Role: PI

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 1

Contribution to Project: He will provide the overall supervision and direct the animal studies.

Change in effort: From 10% to 8% as of June 1, 2015

Name: Adelaide Warsen, MS

Project Role: Research Scientist

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 12

Contribution to Project: Direct performance of all the proposed animal research

Change in effort: None

Name: Modou Mbowe

Project Role: Lab Technician

Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1
Contribution to Project: Research Assistance
Change in effort: Added at 50% as of August 18, 2016

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

No changes

What other organizations were involved as partners?

The collaboration with NWBiospecimen (Univ. of WA), Histology Imaging Core (UW), and Fred Hutchinson Cancer Research Center (FHCRC) Experimental Histopathology Shared Resource is going well. We have not had the results yet, and if there are issues, we expect to resolve them with assistance of Dr. Schmechel (Chief of Pathology at Harborview medical Center and the Director for NWBiospecimen histology core).

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: None

QUAD CHARTS: Attached

9. APPENDICES

None



Topical modulation of the burn wound inflammatory response to improve short- and long term outcomes

2b. Accelerated wound healing
PI: Sam Arbabi, MD, MPH

Award #W81XWH-14-1-0401
Org: University of Washington

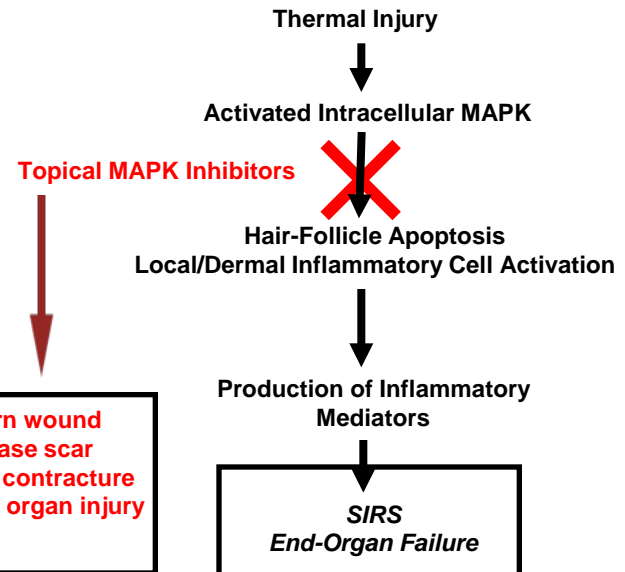
Award Amount: \$1,497,377

Study/Product Aim(s)

- The magnitude and impact of burns associated with warfare can be devastating. We employ a gel formulation of a powerful p38MAPK inhibitor that is ideal for early topical intervention on the battlefield, ready to use, and can be rapidly applied to wounds by self, buddy aid, and/or first responders. This topical treatment would aid in preservation and stabilization of systemic homeostasis, mitigating short and long term deleterious consequences of severe wound injuries, such as secondary organ damage, scar formation, and burn contracture.

Approach

- In the current application, we propose to continue our wound healing studies in an animal burn model that resembles human wound healing. We will use the red Duroc pig burn wound model that resembles human wound healing and scar formation.



1- Improve burn wound healing, decrease scar formation and contracture
2- Reduce end organ injury and mortality.

Timeline and Cost

Activities	FY	14-15	15-16	16-17	17-18
Aim 1: Determine the effect of topical p38MAPK inhibition on wound healing gene expression in the female red Duroc pig model of burn injury					
Aim 2: Determine the effect of topical p38MAPK inhibition on early wound healing post-burn injury					
Aim 3: Define the long-term wound outcome of topical p38MAPK inhibition post-burn injury.					
Estimated Budget (\$K)		367	373	377	381

Updated: October 2016

Goals/Milestones

FY 14-15 Goals –

- ☒ Establish the red Duroc pig burn model

FY 15-16 Goals–

- ☒ Define the inflammatory signaling post burn injury
- ☒ Identify the optimal timing and duration of treatment for p38MAPK.

FY16-17Goals –

- ☐ Demonstrate early wound healing, decreased burn wound depth, and reduced inflammatory response with topical p38MAPK inhibition.

FY 17-18 Goal –

- ☐ Demonstrate decreased scar formation with topical p38 MAPK inhibition.

Comments/Challenges/Issues/Concerns

- If timelines change, comment here.

Budget Expenditure to Date

Projected Expenditure: \$190,000

Actual Expenditure: \$187,074